FILE 'CAPLUS' ENTERED AT 15:35:27 ON 10 NOV 2003

=> S RESTRICTION (W) (ENDONUCLEASE OR ENZYME);S MUTAGEN?;S MUTAT?
         87427 RESTRICTION
         11614 RESTRICTIONS
         98288 RESTRICTION
                 (RESTRICTION OR RESTRICTIONS)
         24293 ENDONUCLEASE
          7512 ENDONUCLEASES
         28333 ENDONUCLEASE
                 (ENDONUCLEASE OR ENDONUCLEASES)
        688961 ENZYME
        397087 ENZYMES
        867102 ENZYME
                 (ENZYME OR ENZYMES)
L1   28959 RESTRICTION (W) (ENDONUCLEASE OR ENZYME)

L2   91791 MUTAGEN?

L3   256267 MUTAT?

=> S (L2,L3) AND L1
L4   4713 ((L2 OR L3)) AND L1

=> S RECOGNITION;S SEQUENCE;S ALTERED
         91451 RECOGNITION
           151 RECOGNITIONS
L5   91520 RECOGNITION
                 (RECOGNITION OR RECOGNITIONS)

        567747 SEQUENCE
        407680 SEQUENCES
L6   675274 SEQUENCE
                 (SEQUENCE OR SEQUENCES)

L7   168330 ALTERED

=> S (L2,L3)(4A)L1
L8   588 ((L2 OR L3))(4A)L1

=> S (L2,L3)(2A)L1
L9   312 ((L2 OR L3))(2A)L1

=> S L5(W)L6;S L7(W)L10
L10   3381 L5(W)L6

L11   8 L7(W)L10

=> D 1-8 CBIB ABS

L11 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
2002:119325 Document No. 136:178936 Process for producing a polynucleotide encoding a restriction endonuclease. Janulaitis, Arvydas; Rimseliene, Renata; Lubys, Arvydas (Fermentas Ab, Lithuania). Eur. Pat. Appl. EP 1179596 A1 20020213, 44 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 2001-305859 20010706. PRIORITY: GB 2000-19744 20000810.

AB A process for producing a polynucleotide encoding a restriction endonuclease with an altered specificity. The process comprises of mutagenizing a polynucleotide encoding a restriction endonuclease with specificity for a recognition sequence so as to produce one or more mutated polynucleotides. The invention also relates to the isolation of polynucleotide encoding a mutated restriction endonuclease with specificity for an ***altered*** ***recognition*** ***sequence*** by selecting a polynucleotide which expresses a

restriction endonuclease with methylase specificity for the ***altered*** ***recognition*** ***sequence*** .

L11 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
1995:680022 Document No. 123:136929 Interaction of the MvaI and SsoII methyltransferases with DNAs altered at the central base pair of the recognition sequence. Brevnov, Maxim G.; Kubareva, Elena A.; Romanova, Elena A.; Volkov, Evgeniy M.; Karyagina, Anna S.; Nikolskaya, Irina I.; Gromova, Elizaveta S. (A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, 119899, Russia). Gene, 157(1/2), 149-52 (English) 1995. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier.
AB The interaction of the MvaI and SsoII DNA methyltransferases (MTases; M.cntdot.MvaI and M.cntdot.SsoII, resp.) with a set of synthetic DNA duplexes, contg. a M.cntdot.MvaI and M.cntdot.SsoII recognition site (CCWGG), was investigated. In these DNA duplexes dA or dT of the recognition site was replaced by nucleoside analogs with modified sugar moieties and heterocyclic bases (2'-deoxy-2'-fluorouridine (flU), 1-(.beta.-D-2'-deoxy-threo-pentofuranosyl)thymine (xT), 1-(.beta.-D-3'-deoxy-threo-pentofuranosyl)uracil (tU)), or by 1,3-propanediol (Prd). A new approach for monitoring methylation of each strand of DNA duplexes by MTases was developed. It allowed the detn. of the influence of the modification in one DNA strand on the methylation of the other. In most cases, for both M.cntdot.MvaI and M.cntdot.SsoII, sugar analog-contg. duplexes showed inhibition of methylation of only the modified strand. Prd-contg. DNA duplexes were not substrates for M.cntdot.MvaI. M.cntdot.SsoII did not methylate DNA duplexes in which the dT residue was replaced by Prd.

L11 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
1995:598194 Document No. 123:30954 Transplantation of a 17-amino acid .alpha.-helical DNA-binding domain into an antibody molecule confers sequence-dependent DNA recognition. McLane, Kathryn E.; Burton, Dennis R.; Ghazal, Peter (Dep. Immunol. Mol. Biol. Neuropharmacol., Scripps Res. Inst., La Jolla, CA, 92037, USA). Proceedings of the National Academy of Sciences of the United States of America, 92(11), 5214-18 (English) 1995. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.
AB Recombinant antibodies capable of sequence-specific interactions with nucleic acids represent a class of DNA- and RNA-binding proteins with potential for broad application in basic research and medicine. The authors describe the rational design of a DNA-binding antibody, Fab-Ebox, by replacing a variable segment of the Ig heavy chain with a 17-amino acid domain derived from TFEB, a class B basic helix-loop-helix protein. DNA-binding activity was studied by electrophoretic mobility-shift assays in which Fab-Ebox was shown to form a specific complex with DNA contg. the TFEB recognition motif (CACGTG). Similarities were found in the abilities of TFEB and Fab-Ebox to discriminate between oligodeoxyribonucleotides contg. ***altered*** ***recognition*** ***sequences*** . Comparable interference of binding by methylation of cytosine residues indicated that Fab-Ebox and TFEB both contact DNA through interactions along the major groove of double-stranded DNA. The results of this study indicate that DNA-binding antibodies of high specificity can be developed by using the modular nature of both Igs and transcription factors.

L11 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
1993:539666 Document No. 119:139666 Purine 8-substitution modulates the recognition by restriction endodeoxyribonulcease EcoRI of octadeoxyribonucleotides (dGGAATTCC). Komatsu, Hiroshi; Kim, Sang Gug; Sakabe, Ichiro; Ichikawa, Takashi; Nakai, Michiaki; Takaku, Hiroshi (Dep. Ind. Chem., Chiba Inst. Technol., Narashino, 275, Japan). Bioorganic & Medicinal Chemistry Letters, 2(6), 565-70 (English) 1992. CODEN: BMCLE8. ISSN: 0960-894X.
AB Octadeoxyribonucleotide with the sequence d(GGA*ATTCC), d(GGAA*TTCC), and d(GG*AATTCC) contg. modified base moieties which have an isosterically ***altered*** ***recognition*** ***sequence*** of the restriction endodeoxyribonuclease ECoRI. The oligomers, with replacement to deoxy-7,8-dihydroasenosine-8-one (dAOH), 8-methoxydeoxyadenosine (dAOMe) and 8-methoxydeoxyguanosine (dGOMe) from deoxyadenosine or deoxyguanosine were used to study their cleavage by the restriction endodeoxyribonuclease EcoRI of the modified oligomers were perfectly resisted compared to d(GGTTAACC). These results suggest that changes in

the base sugar torsion angles of oligomer may modulate recognition by EcoRI.

L11 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
1992:152283 Document No. 116:152283 Synthesis of protected 8-substituted deoxyribonucleosides and its helix stability in oligodeoxyribonucleotides containing the Eco RI recognition site. Komatsu, Hiroshi; Ichikawa, Takashi; Nakai, Michiaki; Takaku, Hiroshi (Dep. Ind. Chem., Chiba Inst. Technol., Narashino, 275, Japan). Nucleosides & Nucleotides, 11(1), 85-95 (English) 1992. CODEN: NUNUD5. ISSN: 0732-8311.
AB Octadeoxyribonucleotides with the sequences of d(GGXATTCC) (I), d(GGAXTTCC) (II, X = deoxy-7,8-dihydroadenosine-8-one, 8-methoxydeoxyadenosine), and d(GX1AATTCC) (III, X1 = 8-methoxydeoxyguanosine) have been prepd. by solid phase synthesis using H-phosphonate units contg. the modified base moieties. These oligomers have an isosterically ***altered*** ***recognition*** ***sequence*** of the restriction endodeoxyribonuclease Eco RI. These oligomers were used for studying recognition phenomena at the functional group level. Thermodn. data show that I (X = 8-methoxydeoxyadenosine) destabilizes such duplexes less strongly than II or III. Further, I-III resisted hydrolysis by Eco RI.

L11 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
1989:510160 Document No. 111:110160 Alteration of a single nucleotide allows efficient binding of H2TF1/KBF1 to the immunoglobulin .kappa. enhancer B motif. Mauxion, Fabienne; Sen, Ranjan (Dep. Biol., Brandeis Univ., Waltham, MA, 02254-9110, USA). Molecular and Cellular Biology, 9(8), 3548-52 (English) 1989. CODEN: MCEBD4. ISSN: 0270-7306.
AB NF-.kappa.B (a protein present constitutively only in B cells) and H2TF1/KBF1 (a more ubiquitously distributed protein[s]) are two transcription factors that recognize very similar DNA sequences. However, the binding site assocd. with the .kappa. Ig gene enhancer (.kappa.B) is recognized predominantly by NF-.kappa.B. Using synthetically ***altered*** ***recognition*** ***sequences*** , it is shown that the B-cell-specific NF-.kappa.B-binding site in the .kappa. enhancer can be converted to one that binds both NF-.kappa.B and the ubiquitous protein(s) H2TF1/KBF1 by substitution of a single nucleotide. Furthermore, transient transfection expts. suggested that NF-.kappa.B and H2TF1/KBF1 are functionally different, even though their DNA recognition specificities are very similar.

L11 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
1986:402628 Document No. 105:2628 Palindromic oligonucleotides containing 7-deaza-2'-deoxyguanosine: solid-phase synthesis of d[(p)GG*AATTCC] octamers and recognition by the endodeoxyribonuclease EcoRI. Seela, Frank; Driller, Hansjuergen (Lab. Org. Bioorg. Chem., Univ. Osnabrueck, Osnabrueck, D-4500, Fed. Rep. Ger.). Nucleic Acids Research, 14(5), 2319-32 (English) 1986. CODEN: NARHAD. ISSN: 0305-1048.
AB Octadeoxynucleotides with the sequence d[(p)GG*AATTCC] were prepd. by solid-phase synthesis employing regular and base-modified phosphoramidites. These oligomers, which contain an isosterically ***altered*** ***recognition*** ***sequence*** of the endodeoxyribonuclease EcoRI, form duplexes under appropriate salt conditions. Since G* can represent 7-deaza-2'-deoxyguanosine, the oligomers were used as probes to study their cleavage by EcoRI. The enzymic hydrolysis of the modified octamer was strongly decreased compared to the regular DNA fragment. This shows that guanine N-7 located at the cleavage site is important for the recognition process by the enzyme. The residual enzymic activity is discussed on the basis of reduced specificity towards the recognition fragment. The fact that this cleavage occurs already under regular conditions indicates that the process described here is based on an intrinsic property of the oligomer and is different from the star activity.

L11 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
1980:71296 Document No. 92:71296 Sequence and sequence variation within the 1.688 g/cm3 satellite DNA of Drosophila melanogaster. Hsieh, Tao-Shih; Brutlag, Douglas (Sch. Med., Stanford Univ., Stanford, CA, 94305, USA). Journal of Molecular Biology, 135(2), 465-81 (English) 1979. CODEN: JMOBAK. ISSN: 0022-2836.
AB The complete nucleotide sequence of the monomer repeating unit of the

1.688 g/cm3 satellite DNA from D. melanogaster was detd. This satellite DNA, which makes up 4% of the Drosophila genome and is located primarily on the sex chromosomes, had a repeat unit 359 base pairs in length. This complex sequence was unrelated to the other 3 major satellite DNAs present in this species, each of which contained a very short repeated sequence only 5-10 base pairs long. The repeated sequence was more similar to the complex repeating units in satellites of mammalian origin in that it contained runs of adenylate and thymidylate residues. The nature of the sequence variations in this DNA was detd. by restriction nuclease cleavage and by direct sequence detn. of (1) individual monomer units cloned in hybrid plasmids, (2) mixts. of adjacent monomers from a cloned segment of this satellite DNA, and (3) mixts. of monomer units isolated by restriction nuclease cleavage of total 1.688 g/cm3 satellite DNA. Both direct sequence detn. and restriction nuclease cleavage indicated that certain positions in the repeat can be highly variable with .ltoreq.50% of certain restriction sites having ***altered*** ***recognition*** ***sequences*** . Despite the high degree of variation at certain sites, most positions in the sequence were highly conserved. Sequence anal. of a mixt. of 15 adjacent monomer units detected only 9 variable positions out of 359 base pairs. Total satellite DNA showed only 4 addnl. positions. Whereas some variability could have been missed due to the sequencing methods used, the variation from 1 repeat to the next is not considered to be random and most of the satellite repeat is conserved. This conservation may reflect functional aspects of the repeated DNA, as part of this sequence has been shown to serve as a binding site for a sequence-specific DNA binding protein isolated from Drosophila embryos.

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=> S L4 AND L10
L12         125 L4 AND L10

=> S L8 AND L12
L13          32 L8 AND L12

=> S L8 AND L10
L14          32 L8 AND L10

=> S L9 AND L10
L15          18 L9 AND L10

=> S L15 NOT L11
L16          17 L15 NOT L11

=> D 1-17 CBIB ABS
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L16 ANSWER 1 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
2002:977271 Document No. 138:232421 PCR designer for restriction analysis of various types of sequence mutation. Ke, Xiayi; Collins, Andrew; Ye, Shu (Wellcome Trust Centre For Human Genetics, University of Oxford, Oxford, OX3 7BN, UK). Bioinformatics, 18(12), 1688-1689 (English) 2002. CODEN: BOINFP. ISSN: 1367-4803. Publisher: Oxford University Press.
AB Restriction anal. is widely used to detect gene mutations such as insertions, deletions and single nucleotide polymorphisms (SNPs). Although such mutation sites sometimes present some natural restriction sites to differentiate the wild-type and mutant sequences, mismatches are often needed in order to create artificial restriction fragment length polymorphisms (RFLPs). In this report, a computer program is described that screens for suitable restriction enzymes, introducing mismatches where appropriate and when necessary, designs primers using the information of the selected restriction enzymes, their ***recognition*** ***sequence*** and locations as well as the information about the mismatches if any. The program, supported by a WWW web interface, is intended to be used online.

L16 ANSWER 2 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
2002:403841 Document No. 137:1491 Engineering nicking endonucleases from type IIs ***restriction*** ***endonuclease*** by ***mutating*** dimerization/cleavage domain. Kong, Huimin; Besnier, Caroline; Xu, Yan (New England Biolabs, Inc., USA). U.S. US 6395523 B1 20020528, 20 pp. (English). CODEN: USXXAM. APPLICATION: US 2001-872861 20010601.
AB The present invention relates to methods to engineer nicking endonucleases

from existing Type IIs restriction endonuclease MlyI and AlwI, and the prodn. of the engineered nicking endonucleases. Specifically, the method comprises identifying a suitable double-stranded nuclease followed by site-directed mutation of the dimerization interface responsible for double-stranded cleavage such that the mutated nuclease cleaves only one DNA strand at a specific location within or adjacent the ***recognition*** ***sequence*** . The mutation occurs by substituting one or more amino acid residues required for dimerization/cleavage, or swapping or substituting the region contg. the dimerization interface with a natural occurring nicking endonuclease, N.BstNBI.

L16 ANSWER 3 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
2002:293886 Document No. 136:321673 Mutation analysis using labeled probe hybridization and nuclease cleavage. Yamamoto, Takeshi; Ikeda, Masafumi (International Reagents Corporation, Japan). PCT Int. Appl. WO 2002031193 A1 20020418, 57 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (Japanese). CODEN: PIXXD2. APPLICATION: WO 2001-JP8882 20011010. PRIORITY: JP 2000-311294 20001011; JP 2001-112359 20010411; JP 2001-286700 20010920.
AB A method and an assay kit for detecting a genetic ***mutation*** , a ***restriction*** ***enzyme*** - ***recognition*** ***sequence*** , or detecting or quantifying the amplification of a specific sequence by in vitro reactions in a homogeneous assay system, without resorting to complicated procedures such as electrophoresis or so-called Bound/Free sepn., are provided. A probe contg. a signal generating agent is hybridized with a target nucleic acid and then treated with an endonuclease and/or an exonuclease or a protein having an exonuclease activity to thereby release the signal generating agent. Use of 2-aminopurine contg. DNA probes and lambda exonuclease, T4 endonuclease VII, or Werner Syndrome protein (WRN) for detection of mismatch mutations is demonstrated.

L16 ANSWER 4 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
1999:533053 Document No. 131:332786 Highly sensitive mutation screening by REF with low concentrations of urea: a blinded analysis of a 2-kb region of the p53 gene reveals two common haplotypes. Feng, Jinong; Buzin, Carolyn H.; Tang, Shih-Huey E.; Scaringe, William A.; Sommer, Steve S. (Department of Molecular Genetics, City of Hope National Medical Center and Beckman Research Institute, Duarte, CA, USA). Human Mutation, 14(2), 175-180 (English) 1999. CODEN: HUMUE3. ISSN: 1059-7794. Publisher: Wiley-Liss, Inc..
AB Restriction endonuclease fingerprinting (REF), a hybrid modification of single-strand conformation polymorphism (SSCP) and restriction endonuclease digestion, has been used previously to detect mutations in 1- to 2-kb segments of DNA. This paper demonstrates that fragment resoln., and thus sensitivity of REF, can be markedly improved by electrophoresis under partially denaturing, rather than nondenaturing, conditions, for genes with a high G+C content. A 2.1-kb segment of the p53 tumor suppressor gene (54.5% G+C) contg. exons 5-9, including the intervening introns, was screened in a blinded anal. of 48 samples from human breast tumors contg. known wild-type or mutant p53 genes. In gels contg. 0.5 M urea, 97% of the mutant samples were detected correctly, and more than 80% of the mutations were localized within a 200-bp region. In the process of this methodol. anal., it was discovered that: (1) there are two common and four uncommon haplotypes; (2) the two common haplotypes occurred in the three races examd., suggesting an ancient origin; and (3) haplotype II is of substantially higher frequency in the Chinese relative to Japanese (P = 0.023) and Caucasians (P = 0.005). Two other improvements in the REF procedure included (1) the selection of an optimal set of restriction endonucleases by new software (REF Select) developed recently in our lab.; and (2) the addn. of an oligonucleotide "tail," contg. two ***recognition*** ***sequences*** for restriction endonucleases, to the PCR primers to prevent coterminal fragments at the end of amplified products. These modifications facilitate the use of REF for efficient and

sensitive mutation screening in p53 and other genes with a high G+C content.

L16 ANSWER 5 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
1996:233444 Document No. 124:282889 Influence of the phosphate backbone on the recognition and hydrolysis of DNA by the EcoRV restriction endonuclease. A study using oligodeoxynucleotide phosphorothioates. Thorogood, Harry; Grasby, Jane A.; Connolly, Bernard A. (Dep. Biochem. Genet., Univ. Newcastle, Newcastle upon Tyne, NE2 4HH, UK). Journal of Biological Chemistry, 271(15), 8855-62 (English) 1996. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB A set of phosphorothioate-contg. oligonucleotides based on pGACGATATCGTC, a self-complementary dodecamer that contains the EcoRV ***recognition*** ***sequence*** (GATATC), has been prepd. The phosphorothioate group has been individually introduced at the central nine phosphate positions and the two diastereomers produced at each site sepd. and purified. The Km and Vmax values found for each of these modified DNA mols. with the EcoRV restriction endonuclease have been detd. and compared with those seen for the unmodified all-phosphate-contg. dodecamer. This has enabled an evaluation of the roles that both of the non-esterified oxygen atoms in the individual phosphates play in DNA binding and hydrolysis by the endonuclease. The results have also been compared with crystal structures of the EcoRV endonuclease, complexed with an oligodeoxynucleotide, to allow further definition of phosphate group function during substrate binding and turnover. For further study, see the related article "Probing the Indirect Readout of the ***Restriction*** ***Enzyme*** EcoRV: ***Mutational*** Anal. of Contacts to the DNA Backbone" (Wenz, A., Jeltsch, A., and Pingoud, A. (1996) J. Biol. Chem. 271, 5565-5573).

L16 ANSWER 6 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
1996:56686 Document No. 124:139506 An isoleucine to leucine mutation that switches the cofactor requirement of the EcoRV restriction endonuclease from magnesium to manganese. Vipond, I. Barry; Moon, Byung-Jo; Halford, Stephen E. (Centre for Molecular Recognition, University of Bristol, Bristol, BS8 1TD, UK). Biochemistry, 35(6), 1712-21 (English) 1996. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.
AB The EcoRV restriction endonuclease cleaves DNA at its ***recognition*** ***sequence*** more readily with Mg2+ as the cofactor than with Mn2+, but, at noncognate sequences that differ from the EcoRV site by one base pair, Mn2+ gives higher rates than Mg2+. A mutant of EcoRV, in which an isoleucine near the active site was replaced by leucine, showed the opposite behavior. It had low activity with Mg2+, but, in the presence of Mn2+ ions, it cleaved the recognition site faster than wild-type EcoRV with either Mn2+ or Mg2+. The mutant was also more specific for the ***recognition*** ***sequence*** than the native enzyme: the noncognate DNA cleavages by wild-type EcoRV and Mn2+ were not detected with the mutant. Further mutagenesis showed that the protein required the same acidic residues at its active site as wild-type EcoRV. The Ile.fwdarw.Leu mutation seems to perturb the configuration of the metal-binding ligands at the active site so that the protein has virtually no affinity for Mg2+ yet it can still bind Mn2+ ions; although, the latter only occurs when the protein is at the recognition site. This contrasts to wild-type EcoRV, where Mn2+ ions bind readily to complexes with either cognate and noncognate DNA and only Mg2+ shows the discrimination between the complexes. The structural perturbation is a specific consequence of leucine in place of isoleucine, since mutants with valine or alanine were similar to wild-type EcoRV.

L16 ANSWER 7 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
1994:501631 Document No. 121:101631 Mutagenesis at a Site-Specifically Modified NarI Sequence by Acetylated and Deacetylated Aminofluorene Adducts. Tebbs, Robert S.; Romano, Louis J. (Department of Chemistry, Wayne State University, Detroit, MI, 48202, USA). Biochemistry, 33(30), 8998-9006 (English) 1994. CODEN: BICHAW. ISSN: 0006-2960.
AB A hotspot for mutagenesis by N-acetyl-2-aminofluorene (AAF) was site-specifically modified with 2-aminofluorene (AF) and AAF adducts, and the mutation frequencies and specificities were detd. and compared. Previous work has shown that the presence of an AAF adduct in aNarI sequence (GGCGCC) results a high mutation frequency for a CG double base pair deletion. In the present study, an M13 deriv. was constructed that

contained a NarI ***recognition*** ***sequence*** in the .beta.-galactosidase gene of bacteriophage M13mp9. This deriv. was site-specifically modified with either an AF or an AAF adduct, the products were characterized, and these templates were then transformed into Escherichia coli wild-type strain JM103 or uvrA strain SMH12. The levels and mutation spectra were detd. either with or without SOS induction. It was found that, with SOS functions induced, the measured mutation frequencies were substantially higher in all cases. More importantly, the types of mutations induced by the AAF and AF adducts were very different: AAF adducts induced almost exclusively CG double base deletion mutations, whereas AF adducts gave rise specifically to base-substitution mutations. The AF-derived mutation spectrum included both G to T and G to A mutations. The results are discussed in light of the current views on the relationship between the DNA structure and mutagenesis.

L16 ANSWER 8 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
1993:118236 Document No. 118:118236 ***Mutagenic*** effects of ***restriction*** ***enzymes*** in Chinese hamster cells: evidence for high mutagenicity of Sau3AI at the hprt locus. Kinashi, Yuko; Nagasawa, hatsumi; Little, John B. (Dep. Cancer Biol., Harvard Sch. Public Health, Boston, MA, 02115, USA). Mutation Research, 285(2), 251-7 (English) 1993. CODEN: MUREAV. ISSN: 0027-5107.

AB CHO cells were exposed to seven different restriction endonucleases by electroporation and their cytotoxicity and mutagenicity measured. Cell killing as detd. by a colony formation assay occurred in a concn.-dependent manner for each enzyme. The D0 of the survival curves were: MspI = 24U; AluI = 31U; Sau3 = 106U; HaeIII = 46U; HinfI = 30U; PvuII = 35U; BamHI = 163U. BamHI and Sau3AI were particularly ineffective in cell killing. For the 6-base ***recognition*** ***sequence*** enzymes, PvuII (a blunt-ended cutter) was much more cytotoxic per unit electroporated than BamHI (a sticky-ended cutter). Among the 4-base cutters, Sau3AI and HaeIII were generally less cytotoxic than HinfI or PvuII. Cell killing appeared to depend on the nature of the ***recognition*** ***sequence*** and cutting sites rather than on the cutting frequency. The mutagenic effects of these restriction endonucleases were investigated by measuring the induced frequencies of hprt gene mutations. The mutagenicity of Sau3AI was dramatically higher than the other enzymes, increasing linearly with dose up to 35U. When normalized for survival, the mutagenicity of Sau3AI relative to the other enzymes was even greater. The mutagenic effect of BamHI, which has the same 5' protruding site as Sau3AI, was much lower at similar dose and survival levels. MspI, BamHI, and PvuII which have no recognition sites within the hprt coding sequence were marginally- or non-mutagenic. Based on these results and the distribution of cutting sites within the hprt cDNA for the enzymes studied, the hypothesis is discussed that a region in exon 4 is highly sensitive to the induction of mutants by DNA double-strand breaks.

L16 ANSWER 9 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
1992:525485 Document No. 117:125485 SILMUT: a computer program for the identification of regions suitable for silent ***mutagenesis*** to introduce ***restriction*** ***enzyme*** ***recognition*** ***sequences*** . Shankarappa, Basavaraju; Vijayananda, K.; Ehrlich, Garth D. (Sch. Med., Univ. Pittsburgh, Pittsburgh, PA, USA). BioTechniques, 12(6), 882-4 (English) 1992. CODEN: BTNQDO. ISSN: 0736-6205.

AB A set of IBM-compatible computer programs designed to selectively identify the potential sites for silent mutagenesis within a target DNA sequence is described. This program is based on a novel strategy of identifying amino acid motifs compatible with each restriction site (BioTechniques 12:3820384,1991). The programs can be used to identify the suitability for the introduction of any 6-base nucleic acid sequences, such as restriction enzyme sites in cassette mutagenesis strategies. The Table program generates a table of multiple amino acid motifs for each restriction enzyme, obtained by translating each unique ***recognition*** ***sequence*** in all 3 reading frames. The SILMUT program, which utilizes the features of Table, will further identify the presence of a match between any amino acid motif of each restriction enzyme and the input target sequence. Minor manipulations of the data base files will enable the individual research to identify the

potential for introduction of any 6-base sequences by silent mutagenesis.

L16 ANSWER 10 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
1992:505262 Document No. 117:105262 'Stop-codon-specific' restriction endonucleases: their use in mapping and gene manipulation. Rowland, Geoffrey C.; Lim, Ping Ping; Glass, Robert E. (Med. Sch., Univ. Nottingham, Nottingham, NG7 2UH, UK). Gene, 116(1), 21-6 (English) 1992. CODEN: GENED6. ISSN: 0378-1119.
AB Certain restriction endonucleases recognize target sequences that contain the stop triplet TAG and are commonly either 4 or 6 bp in length. Interestingly, these restriction targets do not occur at the frequency expected on the basis of base compn. and size. For example, the tetranucleotide MaeI ***recognition*** ***sequence*** (CTAG) occurs considerably less commonly (5-8-fold) in the genome of Escherichia coli (and many other eubacteria) than expected from mononucleotide frequencies. This surprising rarity is particularly evident in protein-encoding genes and is largely dictated by codon usage. Thus, amber (TAG) nonsense mutations frequently give rise to novel MaeI (CTAG) sites which are unique within a translated region. Such amber/MaeI sites, whether arising spontaneously or created in vitro by site-directed mutagenesis, act as a useful phys. marker for the presence of the nonsense mutation and are a convenient startpoint for a range of diverse procedures. These features provide a useful supplement to protein engineering methods which use nonsense suppression to mediate amino acid replacements.

L16 ANSWER 11 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
1992:465469 Document No. 117:65469 Correlation between insertion mutant activities and amino acid sequence identities of the TaqI and TthHB8 restriction endonucleases. Barany, Francis; Zebala, John (Med. Coll., Cornell Univ., New York, NY, 10021, USA). Gene, 112(1), 13-20 (English) 1992. CODEN: GENED6. ISSN: 0378-1119.
AB A two-codon insertion mutagenesis method has been generalized. Over 24 insertion mutants throughout the gene encoding TaqI restriction endonuclease were constructed and activity was characterized. All mutants with activity either cleaved or nicked the canonical T.dwnarw.CGA ***recognition*** ***sequence*** . Some insertion mutants created duplication of gene regions, termed Gemini proteins, which still retained activity. The correlation between mutants with poor activity and the regions of shared amino acid identity between the isoschizomeric TaqI and TthHB8I suggests these regions are involved in DNA recognition and/or catalysis.

L16 ANSWER 12 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
1992:463885 Document No. 117:63885 Analysis of sequence specificity of 5-bromodeoxyuridine-induced reversion in cells containing multiple copies of a mutant gpt gene. Rotstein, Joel B.; Kresnak, Mark T.; Samadashwily, Georgy M.; Davidson, Richard L. (Coll. Med., Univ. Illinois, Chicago, IL, 60612, USA). Somatic Cell and Molecular Genetics, 18(2), 179-88 (English) 1992. CODEN: SCMGDN. ISSN: 0740-7750.
AB For studies on mol. mechanisms of mutagenesis, it would be advantageous to transfer mutants genes with specific alterations into mammalian cells and use the transformed cells in reversion analyses. In the present paper, an efficient method is described for analyzing reversion events occurring in cells that possess multiple copies of a mutational target gene. This method involves amplification of the chromosomally integrated target genes with the PCR and restriction endonuclease digestion of the amplified product. Single reversion events that either create or destroy restriction endonuclease ***recognition*** ***sequences*** that encompass the site of the original mutation can be identified in a background of 10-20 copies of the gene that retain the mutant sequence. This method was used to analyze revertants induced by 5-bromodeoxyuridine (BrdU) in a Chinese hamster ovary cell line that possesses multiple copies of a mutant bacterial gpt gene contg. a specific alteration. The results of this study not only demonstrate the effectiveness of this method for analyzing reversion of a single gene copy in transfectants possessing multiple copies of a mutant target gene, but also demonstrate that the sequence specificity for BrdU-induced mutations is the same in Chinese hamster cells as previously obsd. with mouse cells.

L16 ANSWER 13 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN

1992:253558 Document No. 116:253558 Potentiation of DNA damage by inhibition of poly(ADP-ribosyl)ation: a test of the hypothesis for random nuclease action. Rufer, Joan T.; Morgan, William F. (Lab. Radiobiol. Environ. Health, Univ. California, San Francisco, CA, 94143-0750, USA). Experimental Cell Research, 200(2), 506-12 (English) 1992. CODEN: ECREAL. ISSN: 0014-4827.

AB Pol(ADP-ribosyl)ation is a cellular response to DNA strand breaks by which a large array of proteins becomes covalently modified for a brief period during the lifetime of the DNA breaks. Inhibition of poly(ADP-ribose) polymerase by 3-aminobenzamide after many types of DNA damage leads to a marked increase in DNA strand breakage, repair replication, cytogenetic damage, mutagenesis, and cell killing. It has been hypothesized that poly(ADP-ribose) polymerase may modify potentially degradative endogenous nucleases that can reduce cellular viability. Thus, in the presence of DNA strand breakage, the polymer would bind these enzymes to inhibit their activity. When synthesis of the polymerase is inhibited, the enzymes would act randomly to produce nonspecific damage in the DNA. The authors tested this hypothesis by electroporating restriction enzymes into human cells contg. the shuttle vector pHAZE. Restriction enzymes cleave at specific ***recognition*** ***sequences*** in the lacZ target gene of pHAZE, and mutations result from rejoining errors at the cleavage sites. If the hypothesis were correct, enzyme-treated cells cultured with 3-aminobenzamide to inhibit synthesis of poly(ADP-ribose) polymers would result in a significant increase in ***mutations*** outside the ***restriction*** ***enzyme*** sites. The spectrum of mutations obsd. after electroporation of PvuII (which produces blunt-end double-strand breaks) or PvuI (which produces cohesive-end double-strand breaks) was similar in untreated and 3-aminobenzamide-treated cells. Thus, these results do not support the hypothesis that the increase in damage obsd. when poly(ADP-ribosyl)ation is inhibited is due to a chaotic, nonspecific attack on DNA by endogenous cellular nucleases.

L16 ANSWER 14 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN

1992:122074 Document No. 116:122074 Genotypic analysis of mutations in TaqI restriction recognition sites by restriction fragment length polymorphism/polymerase chain reaction. Sandy, Martha S.; Chiocca, Susanna M.; Cerutti, Peter A. (Dep. Carcinog., Swiss Inst. Exp. Cancer Res., Epalinges/Lausanne, CH-1066, Switz.). Proceedings of the National Academy of Sciences of the United States of America, 89(3), 890-4 (English) 1992. CODEN: PNASA6. ISSN: 0027-8424.

AB Point mutations in somatic cells play a role in the etiol. of several classes of human pathologies. Exptl. procedures are required that allow the detection and quantitation of such mutations in disease-related genes in tissue biopsy samples without the need for the selection of mutated cells. This report describes the genotypic anal. of single base pair mutations in the TaqI endonuclease ***recognition*** ***sequence*** TCGA, residues 2508-2511 of exon 2 of the human c-H-rasI gene, by the restriction fragment length polymorphism/polymerase chain reaction (RFLP/PCR) approach. The high thermostability of TaqI endonuclease allows the continuous removal of eventual residual wild-type sequences during the thermocycling of the PCR and reduces polymerase errors in the final RFLP/PCR product to a min. As few as five copies of a mutant std. contg. two base pair changes in the chosen TaqI site could be rescued from 108 copies of wild-type DNA. TaqI RFLP/PCR holds promise for the monitoring of mutations in biochem. epidemiol.

L16 ANSWER 15 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN

1990:211797 Document No. 112:211797 Heterogeneous alleles and expression of methylmalonyl CoA mutase in mut methylmalonic acidemia. Ledley, Fred D.; Crane, Ana Maria; Lumetta, Michele (Dep. Cell Biol., Baylor Coll. Med., Houston, TX, 77030, USA). American Journal of Human Genetics, 46(3), 539-47 (English) 1990. CODEN: AJHGAG. ISSN: 0002-9297.

AB Methylmalonic acidemia (MMA) can be caused by mutations in the gene coding for the methylmalonyl CoA mutase (MCM) apoenzyme or by mutations in genes required for provision of its adenosylcobalamin cofactor. MCM activity, gene structure, and expression were characterized in a series of primary fibroblast cell lines derived from patients with MCM apoenzyme deficiency. Southern blot anal. reveals normal HindIII and TaqI polymorphisms but no gross insertions, deletions, rearrangements, or point ***mutations*** at ***restriction*** ***endonuclease*** ***recognition*** ***sequences*** . Northern blot anal. demonstrates that several cell

lines have specifically decreased steady-state levels of MCM mRNA. At least 6 independent alleles can be delineated by a haplotype of HindIII and TaqI polymorphisms, the level of mRNA expression, and the biochem. phenotype of the cells. These studies confirm the wide phenotypic spectrum of MMA and provide mol. genetic evidence for a variety of independent alleles underlying this disorder.

L16 ANSWER 16 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
1987:612945 Document No. 107:212945 In vitro sodium bisulfite ***mutagenesis*** of ***restriction*** ***endonuclease*** recognition sites. Merlo, Donald J.; Thompson, David V. (Agrigenet. Adv. Sci. Co., Madison, WI, 53716, USA). Analytical Biochemistry, 163(1), 79-87 (English) 1987. CODEN: ANBCA2. ISSN: 0003-2697.
AB Sodium bisulfite treatment of single-stranded DNA deaminates exposed cytosine residues to form uracil, resulting in cytosine-to-thymidine transition mutations following DNA replication. This reaction was used in vitro to destroy the ***recognition*** ***sequences*** for the restriction endonucleases HindIII and XmaI in the aminoglycoside 3'-phosphotransferase I coding region of plasmid pUC4K. This procedure should be applicable to the mutation of any ***recognition*** ***sequence*** of restriction endonucleases which generate cytosine-contg. single-stranded ends. The possibility of mutagenesis of restriction sites to generate stop codons in coding regions is discussed.

L16 ANSWER 17 OF 17 CAPLUS COPYRIGHT 2003 ACS on STN
1978:472883 Document No. 89:72883 The genome of simian virus 40. 20. Local mutagenesis: A method for generating viral mutants with base substitutions in preselected regions of the viral genome. Shortle, David; Nathans, Daniel (Dep. Microbiol., Johns Hopkins Univ. Sch. Med., Baltimore, MD, USA). Proceedings of the National Academy of Sciences of the United States of America, 75(5), 2170-4 (English) 1978. CODEN: PNASA6. ISSN: 0027-8424.
AB DNA from simian virus 40 (SV40) was prepd. for local mutagenesis by nicking the mol. at a specific site with a restriction endonuclease that recognizes 1 site in SV40 DNA and then extending the nick enzymatically to expose a short, single-stranded segment of DNA. The gapped DNA was treated with a single-strand-specific mutagen, NaHSO3, which converts cytosine to uracil. After mutagenesis, the gap was repaired with DNA polymerase, generating mols. resistant to the restriction enzyme used to make the initial nick. From cells infected with DNA thus modified, SV40 mutants were isolated that had enzyme-resistant genomes. In some cases, precise positions of G-C to A-T transitions could be inferred from the patterns of susceptibility of mutant DNA to other restriction endonucleases whose ***recognition*** ***sequences*** were altered by the mutagenesis procedure. One of the ***restriction*** ***endonuclease*** sites ***mutagenized*** (BglI) maps at the origin of SV40 DNA replications and near sequences corresponding to the 5' ends of viral mRNAs. Many of the resulting BglI-resistant mutants yielded small plaques, suggesting partial defectiveness in DNA replication or transcription.

=> S L13 NOT (L11 OR L16)
L17 14 L13 NOT (L11 OR L16)

=> D 1-14 CBIB ABS

L17 ANSWER 1 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN
2002:846715 Document No. 137:333699 ***Mutagenic*** events in Escherichia coli and mammalian cells generated in response to acetylaminofluorene-derived DNA adducts positioned in the Nar I ***restriction*** ***enzyme*** site. Tan, Xingzhi; Suzuki, Naomi; Grollman, Arthur P.; Shibutani, Shinya (Laboratory of Chemical Biology Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, NY, 11794-8651, USA). Biochemistry, 41(48), 14255-14262 (English) 2002. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.
AB Comparative ***mutagenesis*** studies of N-(2'-deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-AAF) and N-(2'-deoxyguanosin-8-yl)-2-aminofluorene (dG-AF) adducts positioned in the Nar I ***restriction*** ***enzyme*** site were performed using Escherichia coli (E. coli) and

simian kidney (COS-7) cells. Oligodeoxynucleotides (5'TCCTCG1G2CG3CCTCTC) contg. a ***recognition*** ***sequence*** for the Nar I ***restriction*** ***enzyme*** were modified site-specifically with dG-AAF or dG-AF. Modified and unmodified oligomers inserted into single-stranded phagemid shuttle vectors were used to transform E. coli or to transfect COS-7 cells. Following replication in host cells, progeny plasmids were recovered and analyzed for ***mutations*** . In SOS-induced E. coli, dG-AAF primarily induced one- and two-base deletions. The ***mutational*** frequency varied, depending on the position modified in the Nar I site; 91% two-base deletions were obsd. at G3, while 8.4% and 2.8% deletions were detected at G2 and G1, resp. In contrast, dG-AF at any position in the Nar I site failed to produce deletions, generating primarily G .fwdarw. T transversions (***mutational*** frequency, 7.6-8.4%). In COS-7 cells, both dG-AAF and dG-AF primarily induced G .fwdarw. T transversions. ***Mutation*** frequencies for dG-AAF were 9.4-24%, the highest values being at G1 and G3. ***Mutation*** frequencies for dG-AF were 9.3-21%, the higher value at G2. We conclude from this study that the ***mutation*** potential of dG-AAF and dG-AF depends on the structure of the adduct, the sequence context of the lesion, and the host cell used for the expt.

L17 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

2002:391583 Document No. 136:396942 Method using fluorophore-labeled nucleotides, amplification primers, and ***restriction*** ***endonucleases*** for identifying polymorphisms and ***mutations*** by fluorescence polarization. Van Ness, Jeffrey; Galas, David J.; Garrison, Lori K. (Keck Graduate Institute, USA). PCT Int. Appl. WO 2002040126 A2 20020523, 77 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US30743 20011001. PRIORITY: US 2000-PV237409 20001002; US 2000-PV247173 20001110; US 2000-PV247172 20001110; US 2000-PV247275 20001110; US 2000-PV247166 20001110; US 2000-PV247167 20001110; US 2001-PV263971 20010124; US 2001-PV269244 20010215; US 2001-PV300319 20010621; US 2001-PV300350 20010621; US 2001-PV301394 20010627.

AB The invention provides a method for identifying a nucleotide at a defined position in a target nucleic acid using ***restriction*** ***endonucleases*** and fluorescence polarization. The method comprises (a) forming a mixt. of a first oligonucleotide primer (P1), a second oligonucleotide primer (P2), and the target nucleic acid (T). P1 is complementary to T at a position 3' to the defined position and P2 is complementary to the complement of T at a position 3' to the defined position. P1 and P2 comprise a first const. ***recognition*** ***sequence*** (CRS) of a first strand and a second CRS of a second strand of an interrupted ***restriction*** ***endonuclease*** ***recognition*** ***sequence*** (IRERS), resp., but not a complete IRERS, the complete IRERS being a double-stranded nucleic acid having the first and second strands and comprising the first and the second CRS linked by a variable ***recognition*** ***sequence*** (VRS). The method further comprises (b) extending P1 and P2 to form a fragment having the complete IRERS wherein the nucleotide to be identified is within the VRS; (c) cleaving the fragment with a ***restriction*** ***endonuclease*** that recognizes the complete IRERS thereby producing a 5'-overhang consisting of either the nucleotide to be identified or its complement; (d) filling in the 3'-recessed terminus corresponding to the 5'-overhang with a fluorophore-labeled nucleoside triphosphate; and (e) detecting the incorporated fluorophore by fluorescence polarization. The invention further provides compds., compns., and kits related to the method.

L17 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1999:617272 Document No. 131:347175 Needle-in-a-haystack detection and identification of base substitution ***mutations*** in human tissues. Wilson, Vincent L.; Wei, Qi; Wade, Kerry R.; Chisa, Midori; Bailey, Deidre; Kanstrup, Christopher M.; Yin, Xiuqin; Jackson, Chad M.; Thompson,

Barbara; Lee, William R. (Institute of Environmental Studies, Louisiana State University, Baton Rouge, LA, 70803, USA). Mutation Research, 406(2-4), 79-100 (English) 1999. CODEN: MUREAV. ISSN: 0027-5107. Publisher: Elsevier Science B.V..

AB Background and induced germline ***mutagenesis*** and other genotoxicity studies have been hampered by the lack of a sufficiently sensitive technique for detecting ***mutations*** in a small cluster of cells or a single cell in a tissue sample composed of millions of cells. The most frequent type of genetic alteration is intragenic. The vast majority of oncogenic ***mutations*** in human and mammalian cancer involves only single base substitutions. The authors have developed universally applicable techniques that not only provide the necessary sensitivity and specificity for site specific ***mutagenesis*** studies, but also identify the point ***mutation***. The exponential amplification procedures of polymerase chain reaction (PCR) and ligase chain reaction (LCR) have been combined with ***restriction*** ***endonuclease*** (RE) digestion to enable the selective enrichment and detection of single base substitution ***mutations*** in human oncogenic loci at a sensitivity of one mutant in more than 107 wild type alleles. These PCR/RE/LCR procedures have been successfully designed and used for codons 12 and 248 of the Ha-ras and p53 genes, resp., both of which contain a natural MspI ***restriction*** ***endonuclease*** ***recognition*** ***sequence***. These procedures have also been adapted for the detection and identification of ***mutations*** in oncogenic loci that do not contain a natural ***restriction*** ***endonuclease*** ***recognition*** ***sequence***. Using PCR techniques, a HphI site was incorporated into the codons 12/13 region of the human N-ras gene, which was then used for the selective enrichment of mutants at this oncogenic locus. These PCR/RE/LCR procedures for base substitution ***mutations*** in codon 12 of the N-ras gene were found to have the sensitivity of detection of at least one mutant allele in the presence of the DNA equiv. of 106 wild type cells. Only one peripheral blood leukocyte DNA specimen out of nine normal individuals displayed an observable Ha-ras ***mutation*** that was present at frequency between 10-5 and 10-6. These PCR/RE/LCR techniques for detecting and identifying base substitution ***mutations*** are universally applicable to almost any locus or base site within the human or animal genome. With the added advantage of the adjustability of both the amt. of DNA (no. of genomes) to be tested and the sensitivity (10-2 to 10-7) of the assay selection or enrichment procedures, these PCR/RE/LCR techniques will be useful in addressing a broad range of important questions in ***mutagenesis*** and carcinogenesis.

L17 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1998:699996 Document No. 130:62297 Application of the restriction site ***mutation*** technique to N-methyl-N-nitrosourea-induced ***mutations*** in the rat. Suzen, Sinan; Jenkins, Gareth J. S.; Parry, James M. (School of Biological Sciences, University of Wales Swansea, Swansea, SA2 8PP, UK). Teratogenesis, Carcinogenesis, and Mutagenesis, 18(4), 171-182 (English) 1998. CODEN: TCMUD8. ISSN: 0270-3211. Publisher: Wiley-Liss, Inc..

AB The restriction site ***mutation*** (RSM) assay was developed in this lab. for the detection of point ***mutations*** that occur within ***restriction*** ***endonuclease*** ***recognition*** ***sequences*** in the genomic DNA of the rat. ***Mutations*** were detected and identified in a no. of tissues from N-methyl-N-nitrosourea (MNU)-treated rats. Resistant ***restriction*** ***enzyme*** products were detected in 5 of the 13 ***restriction*** ***endonuclease*** ***recognition*** ***sequences*** tested (NcoI, BslI, CfoI, DdeI, and HindIII). These ***mutations*** were detected in the p53 tumor suppressor gene and the H-ras protooncogene. No resistant RSM products were detected in any of the samples taken from untreated animals. The MNU-induced ***mutations*** were identified as G to A and A to G transitions. Our results describe the first successful application of the RSM assay in detecting induced ***mutations*** in the rat and highlight the usefulness of the RSM assay in the anal. of ***mutagen*** -induced base changes without the requirement for selection of a mutant phenotype. Given the increasing use of the rat as an animal model in genotoxicity studies, the development of such tests is essential for future genotoxicity investigations.

L17 ANSWER 5 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN
1996:169068 Document No. 124:221876 A simple method for site-specific ***mutagenesis*** that leaves the rest of the template unaltered. Tomic-Canic, Marjana; Sunjevaric, Ivana; Blumenberg, Miroslav (Med. Cent., New York Univ., New York, NY, USA). Methods in Molecular Biology (Totowa, NJ, United States), 57, 259-67 (English) 1996. CODEN: MMBIED. ISSN: 1064-3745.

AB A method for site-specific ***mutagenesis*** using PCR and restriction digestion with type II ***restriction*** ***endonucleases*** is described. The procedure involves PCR between 2 pairs of primers, designated outside and inside. The inside primers incorporate both the desired ***mutation*** (s) and restriction ***recognition*** ***sequences*** for a type II enzyme. Following in vitro amplification, the 2 PCR products are digested with the designated type II enzyme, thereby removing the assocd. ***recognition*** ***sequences*** from their ends. Subsequent ligation and cloning steps recreate the original template except for the designed ***mutations*** .

L17 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN
1995:680013 Document No. 123:106391 Site-directed ***mutagenesis*** in the catalytic center of the ***restriction*** ***endonuclease*** EcoRI. Grabowski, Gabriele; Jeltsch, Albert; Wolfes, Heiner; Guenter, Maass; Alves, Juergen (Zentrum Biochemie, Medizinische Hochschule Hannover, Hannover, D-30623, Germany). Gene, 157(1/2), 113-18 (English) 1995. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier.

AB The catalytic center of the ***restriction*** ***endonuclease*** (ENase) EcoRI is structurally homologous to that of EcoRV, BamHI and PvuII. Each of these ENases contains a short motif of three to four amino acid (aa) residues which are positioned in a similar orientation to the scissile phosphodiester bond. We have ***mutated*** these aa (Pro90, Asp91, Glu111 and Lys113) in EcoRI to det. their individual roles in catalysis. The replacement of Asp91 and Lys113, resp., by conservative ***mutations*** (Ala91, Asn91, Ala113, Gln113, His113 and Leu113) resulted in a redn. of binding affinity and complete loss of cleavage activity. Only Lys113 .fwdarw. Arg substitution still allows to cleave DNA, albeit with a rate reduced by at least four orders of magnitude. Lys113 seems to stabilize the structure of the wild-type (wt) ENase since all five ENase variants with ***mutations*** at this position show a strongly enhanced tendency to aggregate. The Ala and Gln mutants of Glu111 bind the ***recognition*** ***sequence*** slightly stronger than wt EcoRI and cleave it with a low, but detectable rate. Only the Glu111 .fwdarw. Lys mutant, in which the charge is reversed, shows neither binding no cleavage activity. Pro90 is not important for catalysis, because the Ala90 mutant cleaves DNA with an only slightly reduced rate. Under star conditions, however, this mutant is even more active than wt EcoRI. Therefore, the charged aa Asp91, Glu111 and Lys113 are essential for catalytic activity of the EcoRI ENase. Differences in the individual contributions of these aa to binding and catalysis, as compared with results obtained with EcoRV and BamHI mutants, show that similar catalytic centers are used in a slightly different way by these three ENases.

L17 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN
1994:649437 Document No. 121:249437 Location of putative binding and catalytic sites of NaeI by random ***mutagenesis*** . Holtz, Julia K.; Topal, Michael D. (Dep. Pathology, Univ. North Carolina Med. Sch., Chapel Hill, NC, 27599-7295, USA). Journal of Biological Chemistry, 269(44), 27286-90 (English) 1994. CODEN: JBCHA3. ISSN: 0021-9258.

AB Endonuclease NaeI is a prototype for an unusual group of type II ***restriction*** ***endonucleases*** that must bind two DNA ***recognition*** ***sequences*** to cleave DNA. The naeIR gene, expressed from a Ptac promoter construct, was toxic to Escherichia coli in the absence of NaeI-sequence specific methylases. The naeIR gene was ***mutagenized*** with N-methyl-N'-nitrosoguanidine; four classes of NaeI variants were isolated in the absence of protecting methylase activity. Class I variants (T60I, E70K) lacked detectable cleavage activity, but displayed good sequence-specific DNA binding. Class II variants (D95N, G141D) displayed 1-5% of the wild-type cleavage activity and normal DNA binding. Class III variants (G131E, G131R, G155D, G245E) displayed significantly attenuated cleavage and binding activities. Class IV variants (G197D, G214R/A219T, G236S, L241P, G245E, G245R, G250E, G270E)

lacked both cleavage and binding activities. These results imply two amino acids (Thr-60, Glu-70) essential for catalysis. In addn., two domains are indicated in NaeI: one (Thr-60 to Gly-155) mediates substrate binding and catalysis, the other (Gly-197 to Gly-270) may mediate binding of the activating DNA sequence. The results are compared with the active site residues of EcoRI, EcoRV, and BamHI.

L17 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN
1993:469439 Document No. 119:69439 A directed search for ***mutations*** in hemophilia A using ***restriction*** ***enzyme*** analysis and denaturing gradient gel electrophoresis. A study of seven exons in the factor VIII gene of 170 cases. Lavergne, J. M.; Bahnak, B. R.; Vidaud, M.; Laurian, Y.; Meyer, D. (Hop. Bicetre, Le Kremlin Bicetre, F-94275, Fr.). Nouvelle Revue Francaise d'Hematologie, 34(1), 85-91 (English) 1992. CODEN: NRFHA4. ISSN: 0029-4810.
AB Genomic DNA from 170 unrelated hemophilia A patients was examd. for gene defects in the coding region of the Factor VIII gene. Exons 18, 22-24 and 26 contain a CGA codon for arginine within the ***recognition*** ***sequence*** for the ***restriction*** ***enzyme*** Taq I. These five sites were amplified by the polymerase chain reaction and tested for abnormal Taq I restriction patterns. In five cases, the enzyme Taq I failed to digest the amplified fragments. Direct sequencing of the amplified products demonstrated a C to T transition in the coding strand of exons 18, 22 and 24 in three severe hemophilia A patients resulting in TGA termination codons. Two patients showed G to A transition in exons 24 and 26 reflecting a C to T transition in the non-coding strand substituting a glutamine for an arginine. Three deletions involving exon 26 and one exons 23-26 were found in severe hemophiliac patients. In contrast, exons 23 and 24 failed to amplify in one patient with a moderate form of the disease suggesting an in-frame splicing of exons 22 and 25. Exon 8 and the 3' end of exon 14 were analyzed by denaturing gradient gel electrophoresis (DGGE). Two patients with a moderate form of the disease demonstrated an abnormal electrophoretic pattern in exon 8 and sequencing demonstrated missense ***mutations*** at codon 372 for arginine within a thrombin activation site. One missense ***mutation*** was a C to T transition substituting cysteine for argining and the other was an infrequent G to C transversion at an adjacent nucleotide changing the same arginine to proline. An aberrant DGGE pattern in exon 14 was assocd. with a G to T transversion at codon 1615 for glutamic acid resulting in a TAG termination codon. The search for specific ***mutations*** in these 7 exons demonstrated the causative ***mutation*** in 13 cases (7.6%) of hemophilia A.

L17 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN
1993:186821 Document No. 118:186821 ***Mutational*** analysis of the function of Gln115 in the EcoRI ***restriction*** ***endonuclease*** , a critical amino acid for recognition of the inner thymidine residue in the sequence -GAATTC- and for coupling specific DNA binding to catalysis. Jeltsch, Albert; Alves, Juergen; Oelgeschlaeger, Thomas; Wolfes, Heiner; Maass, Guenter; Pingoud, Alfred (Zent. Biochem., Med. Hochsch. Hannover, Hannover, D-3000/61, Germany). Journal of Molecular Biology, 229(1), 221-34 (English) 1993. CODEN: JMOBAK. ISSN: 0022-2836.
AB The Gln-115 residue of ***restriction*** ***endonuclease*** EcoRI has previously been proposed to form a hydrophobic contact to the Me group of the inner thymidine of the EcoRI ***recognition*** ***sequence*** , -GAATTC-, and to be involved in intramol. H-bonds to the main chain at positions 140 and 143 as well as to the side-chain of Asn-173. Here, Gln-115 was exchanged for Ala and Glu by site-directed ***mutagenesis*** and the purified mutant proteins (Q115A and Q115E) were analyzed biochem. and physicochem. Q115A and Q115E had the same secondary structure compn. as wild-type EcoRI but were less stable toward thermal denaturation than the wild-type enzyme. In contrast to wild-type EcoRI, the mutant proteins showed a biphasic denaturation profile under alk. pH, presumably because the amino acid exchange labilized one part of the mol., which unfolded before the rest of the protein was denatured. Q115A was catalytically inactive under normal buffer conditions, in part due to a diminished affinity toward DNA. At low ionic strength and alk. pH, as well as in the presence of Mn2+, i.e., under conditions where wild-type EcoRI shows a relaxed specificity, Q115A was active, however not as much as wild-type EcoRI. Under these conditions, it cleaved the canonical sequence, -GAATTC-, with the same kcat/Km value as the sequence, -GAAUTC-, which

differed from the former sequence by a single Me group, whereas wild-type EcoRI showed a 10-fold lower kcat/Km for cleavage of -GAAUTC- than for -GAATTC-. Binding expts., carried out in the absence of Mg2+, demonstrated that Q115A had a similar affinity toward -GAATTC- as to -GAAUTC-, whereas wild-type EcoRI bound to -GAATTC- with a 10-fold preference over -GAAUTC-. On the basis of these thermodn. and kinetic results, it was concluded that the hydrophobic contact between the .gamma.-methylene group of Gln-115 and the Me group of the inner thymidine contributed .apprx.3 kJ/mol (.apprx.0.7 kcal/mol) to the energy of interaction, both in the ground and the transition state. Q115E was catalytically inactive under normal buffer conditions, but became active at low ionic strength or in the presence of Mn2+. Different from Q115A, Q115E was inactive at alk. pH and its DNA binding affinity was highest at acidic pH. The dependence of its DNA cleavage activity on pH, which was governed by a pKa of 7.35, could be attributed to the protonation of the newly introduced glutamic acid residue, if it was assumed that the carboxyl group is located in a nonpolar environment and/or involved in a H-bond as the donor. Taken together, these results demonstrated that Gln-115, as suggested on the basis of the revised EcoRI-DNA co-crystal structure, interacts with the inner thymidine of the EcoRI ***recognition*** ***sequence*** and has a structure stabilizing role. In addn., the results suggested that Gln-115 is crucial for coupling specific DNA binding to catalysis under normal buffer conditions and it is suggested that this coupling is achieved because direct and indirect involvement of Gln-115 in base recognition induces local conformational alterations of the C-terminal region of .beta.-strand .beta.3, which contains Lys-113 and Glu-111 that are constituents of the catalytic center of EcoRI. Activation of the catalytic center, therefore, could be triggered by Gln-115.

L17 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1993:117660 Document No. 118:117660 Increased antibody expression from Escherichia coli through wobble-base library ***mutagenesis*** by enzymic inverse PCR. Stemmer, Willem P. C.; Morris, Suzanne K.; Kautzer, Curtis R.; Wilson, Barry S. (Ther. Dep., Hybritech, Inc., San Diego, CA, 92196-9006, USA). Gene, 123(1), 1-7 (English) 1993. CODEN: GENED6. ISSN: 0378-1119.

AB The value of a new library ***mutagenesis*** approach, called library enzymic inverse PCR (LEIPCR), was tested for expression-level enhancement of antibody Fv fragments produced in Escherichia coli. The prodn. level of active, metal chelate-specific antibody was limited by a low expression level of the second, heavy-chain cistron. To increase the prodn. level, LEIPCR was applied to the wobble bases of the second cistron leader peptide. In LEIPCR ***mutagenesis*** , the entire plasmid is amplified using ***mutagenic*** primers with class-IIS ***restriction*** ***endonuclease*** (ENase) sites at their 5' ends. The PCR product is digested with the class-IIS ENase (here, BsaI; GGTCTCN .dwnarw.NNNN.uparw.), which removes its own ***recognition*** ***sequence*** , and the ends are self-ligated. Thus, LEIPCR can be used to make plasmid mutant libraries regardless of the nucleotide sequence, and independent of available ENase sites. The resulting library of 107 wobble mutants was screened for active Fv by a colony filter lift. A selected mutant was shown to produce 4-11-fold more active Fv than the wild type (wt), and 5-fold more heavy chain. ***Mutations*** outside of the leader peptide were shown not to be involved. The ***mutated*** areas of the mRNAs of two different up-mutants may have less secondary structure than the wt. Thus, the sequence of the mRNA of the second leader peptide was limiting to the expression level of heavy-chain and active Fv.

L17 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1992:422441 Document No. 117:22441 EcoRV ***restriction*** ***endonuclease*** : communication between DNA recognition and catalysis. Vermote, Christian L. M.; Vipond, I. Barry; Halford, Stephen E. (Cent. Mol. Recognit., Univ. Bristol, Bristol, BS8 1TD, UK). Biochemistry, 31(26), 6089-97 (English) 1992. CODEN: BICHAW. ISSN: 0006-2960.

AB A genetic system was constructed for the ***mutagenesis*** of the EcoRV ***restriction*** ***endonuclease*** and for the overprodn. of mutant proteins. The system was used to make 2 mutants of EcoRV, with Ala in place of either Asn-185 or Asn-188. In the crystal structure of

the EcoRV-DNA complex, both Asn-185 and Asn-188 contact the DNA within the EcoRV ***recognition*** ***sequence*** . But neither ***mutation*** affected the ability of the protein to bind to DNA. In the absence of metal cation cofactors, the mutants bound DNA with almost the same affinity as that of the wild-type enzyme. In the presence of Mg2+, both mutants retained the ability to cleave DNA specifically at the EcoRV ***recognition*** ***sequence*** , but their activities were severely depressed relative to that of the wild-type. In contrast, with Mn2+ as the cofactor, the mutant enzymes cleaved the EcoRV recognition site with activities that were close to that of the wild-type. When bound to DNA at the EcoRV recognition site, the mutant proteins bound Mn2+ readily, but they had much lower affinities for Mg2+ than the wild-type enzyme. This was the reason for their low activities with Mg2+ as the cofactor. The arrangement of the DNA recognition functions, at one location in the EcoRV ***restriction*** ***enzyme*** , are therefore responsible for organizing the catalytic functions at a sep. location in the protein.

L17 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1991:484921 Document No. 115:84921 Synthesis, ***mutagenicity*** , binding to pBR 322 DNA and antitumor activity of platinum(II) complexes with ethambutol. Coluccia, Mauro; Fanizzi, Francesco P.; Giannini, Giuseppe; Giordano, Domenico; Intini, Francesco P.; Lacidogna, Gaetano; Loseto, Francesco; Mariggio, Maria A.; Nassi, Anna; Natile, Giovanni (Ist. Patol. Gen., Bari, I-70124, Italy). Anticancer Research, 11(1), 281-7 (English) 1991. CODEN: ANTRD4. ISSN: 0250-7005.

AB The platinum complexes of [R,R(-)]-, [S,S(+)]-, and [S,R(.+-.)]-ethambutol were prepd. and their biol. activity tested. All 3 compds. interacted with the DNA ***recognition*** ***sequences*** for the ***restriction*** ***enzymes*** Bam H and Ava I, indicating a binding preference for the GC-rich sequences of DNA. The complex which showed the greatest interaction with guanines {the [S,R(.+-.)]-ethambutol complex} also showed the greatest ***mutagenicity*** . Only the [S,S(+)]-isomer complex showed antitumor activity, and this was not correlated with either ***restriction*** ***enzyme*** -inhibiting activity or with ***mutagenicity*** .

L17 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1984:623816 Document No. 101:223816 Structural alterations of the aprt locus induced by deoxyribonucleoside triphosphate pool imbalances in Chinese hamster ovary cells. Goncalves, Otelinda; Drobetsky, Elliot; Meuth, Mark (Clin. Res. Inst. Montreal, Montreal, QC, H2W 1R7, Can.). Molecular and Cellular Biology, 4(9), 1792-9 (English) 1984. CODEN: MCEBD4. ISSN: 0270-7306.

AB Mutants induced at the adenine phosphoribosyl transferase [9027-80-9] (aprt) locus by dTTP [365-08-2] or dCTP [2056-98-6] pool imbalances were examd. for alterations in genomic DNA sequences. No observable changes were detected by Southern blot anal. of most mutant DNAs, suggesting induction of base pair (bp) alterations or other events below the level of detection (.apprx.30 bp). However, in a few strains (11 from a total collection of 125 mutant cell strains), these events could be localized to ***restriction*** ***endonuclease*** ***recognition*** ***sequences*** when the ***mutations*** resulted in the loss or gain of a particular site. The distribution of lost or gained sites in aprt-deficient mutants induced by the 2 types of pool imbalances clearly varied, with those occurring in a ***mutator*** strain with increased dCTP clustering at 1 end of the aprt gene. Mutants induced by dTTP also revealed novel events: multiple restriction site modifications in a small region of the aprt gene in 1 mutant and a small (.apprx.50 bp) insertion or duplication of DNA sequences. Very few deletion or insertion mutants were detected at the aprt locus. The significance of these findings in terms of the known biochem. and genetic consequences of these imbalances is discussed.

L17 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2003 ACS on STN

1984:152947 Document No. 100:152947 The nucleotide sequence recognized by the Escherichia coli A restriction and modification enzyme. Kroeger, Manfred; Hobom, Gerd (Inst. Biol. III, Univ. Freiburg, Freiburg, D-7800, Fed. Rep. Ger.). Nucleic Acids Research, 12(2), 887-99 (English) 1984. CODEN: NARHAD. ISSN: 0305-1048.

AB He nucleotide ***recognition*** ***sequence*** for the

restriction-modification enzyme EcoA of E. coli A has been detd. to be GAG-7N-GTCA (N = nucleotide). This sequence is fairly similar to, but distinctly different from, the 2 other type I ***restriction*** ***enzyme*** recognition sites known for E. coli B and E. coli K12, resp. N6-adenosine methylation has been obsd. at nucleotide positions 2 and 12 within the sequence after modification by EcoA. As a ref. point for mapping the single EcoA site in phage .lambda., the position of .lambda. point ***mutation*** Oam29 has also been detd.

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=> E SAMUELSON J/AU
=> S E3,E4,E6-E8
             7 "SAMUELSON J"/AU
             1 "SAMUELSON J C"/AU
             1 "SAMUELSON JAMES"/AU
             8 "SAMUELSON JAMES C"/AU
             1 "SAMUELSON JAMES CHRISTOPHER"/AU
L18         18 ("SAMUELSON J"/AU OR "SAMUELSON J C"/AU OR "SAMUELSON JAMES"/AU
               OR "SAMUELSON JAMES C"/AU OR "SAMUELSON JAMES CHRISTOPHER"/AU)

=> S L18 AND L1
L19          3 L18 AND L1

=> S L19 NOT (L11,L16,L17)
L20          3 L19 NOT ((L11 OR L16 OR L17))

=> D 1-3 CBIB ABS
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L20 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
2003:571173 Document No. 139:113669 Alteration of ***restriction*** ***endonuclease*** specificity by genetic selection. ***Samuelson,*** *** James C.*** ; Xu, Shuang-Yong (New England Biolabs, Inc., USA). PCT Int. Appl. WO 2003060152 A2 20030724, 51 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US542 20030109. PRIORITY: US 2002-PV347403 20020110.

AB Methods and compns. are provided for altering the DNA recognition and cleavage characteristics of an endonuclease without prior knowledge of the endonuclease's three-dimensional structure and/or amino acid residues responsible for activity and/or specificity. A protocol developed to achieve in vivo selection process includes one or more of the following steps: (1) generating a mutated endonuclease library within an expression vector or plasmid; (2) introducing the endonuclease library into prokaryotic host cells pre-modified with a non-cognate pattern of methylation; (3) pooling survivors and plasmid DNA from the cells; (4) isolating active endonuclease clones by culturing individual colonies for a short time at a low temp. and prepg. plasmid DNA from these cultures; (5) introducing individual plasmid isolates (or pooled plasmid DNA) into a DNA damage indicator strain which is pre-modified with the same pattern of methylation as in step (2). The stringent selection method allows rapid screening of an estd. 107 variants in one round. The method is exemplified by increasing the substrate specificity of Bacillus stearothermophilus Y406 endonuclease BstYI (5'-RGATCY-3') to single site recognition (5'-AGATCT-3').

L20 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
2002:482232 Document No. 137:212814 Directed evolution of ***restriction*** ***endonuclease*** BstYI to achieve increased substrate specificity. ***Samuelson, James C.*** ; Xu, Shuang-yong (New England Biolabs, Beverly, MA, 01915, USA). Journal of Molecular Biology, 319(3), 673-683 (English) 2002. CODEN: JMOBAK. ISSN: 0022-2836. Publisher: Elsevier Science Ltd..

AB ***Restriction*** ***endonucleases*** have proven to be esp. resistant to engineering altered substrate specificity, in part, due to the requirement of a cognate DNA methyltransferase for cellular DNA

protection. Thermophilic ***restriction*** ***endonuclease*** BstYI recognizes and cleaves all hexanucleotide sequences described by 5'-R.dwnarw.GATCY-3' (where R = A or G and Y = C or T). The recognition of a degenerate sequence is a relatively common feature of the >3000 characterized ***restriction*** ***endonucleases*** . . However, very little is known concerning substrate recognition by these enzymes. The authors' objective was to investigate the substrate specificity of BstYI by attempting to increase the specificity to recognition of only AGATCT. By a novel genetic selection/screening process, 2 BstYI variants were isolated with a preference for AGATCT cleavage. A fundamental element of the selection process is modification of the Escherichia coli host genomic DNA by the BglII N4-cytosine methyltransferase to protect AGATCT sites. The amino acid substitutions resulting in a partial change of specificity were identified and combined into one superior variant designated NN1. BstYI variant NN1 displayed a 12-fold preference for cleavage of AGATCT over AGATCC or GGATCT. Moreover, cleavage of the GGATCC sequence was no longer detected. This study provides further evidence that lab. evolution strategies offer a powerful alternative to structure-guided protein design.

L20 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN

2002:444432 Document No. 137:16511 Method for cloning, and expression in E. coli, and purification of BstYI ***restriction*** ***endonuclease*** and BstYI methylase from Bacillus stearothermophilus Y406. Xu, Shuang-Yong; ***Samuelson, James*** ; Pelletier, John; Sibley, Marion; Wilson, Geoffrey G. (New England Biolabs, Inc., USA). U.S. US 6403354 B1 20020611, 18 pp. (English). CODEN: USXXAM. APPLICATION: US 2001-766055 20010119.

AB The present invention relates to a method for cloning the BstYI ***restriction*** ***endonuclease*** from Bacillus stearothermophilus into E. coli by methylase selection and inverse PCR amplification of the adjacent DNA. A methylase gene with high homol. to amino-methyltransferases (N4-cytosine methylases) was found in a DNA library after methylase selection. This gene was named BstYI methylase gene (bstYIM). The present invention relates to recombinant DNA which encodes the BstYI ***restriction*** ***endonuclease*** as well as BstYI methyltransferase, expression of BstYI ***restriction*** ***endonuclease*** and M.BstYI in E. coli cells contg. the recombinant DNA. It also relates to methods for purifn. of the recombinant BstYI ***restriction*** ***endonuclease*** and BstYI methyltransferase.